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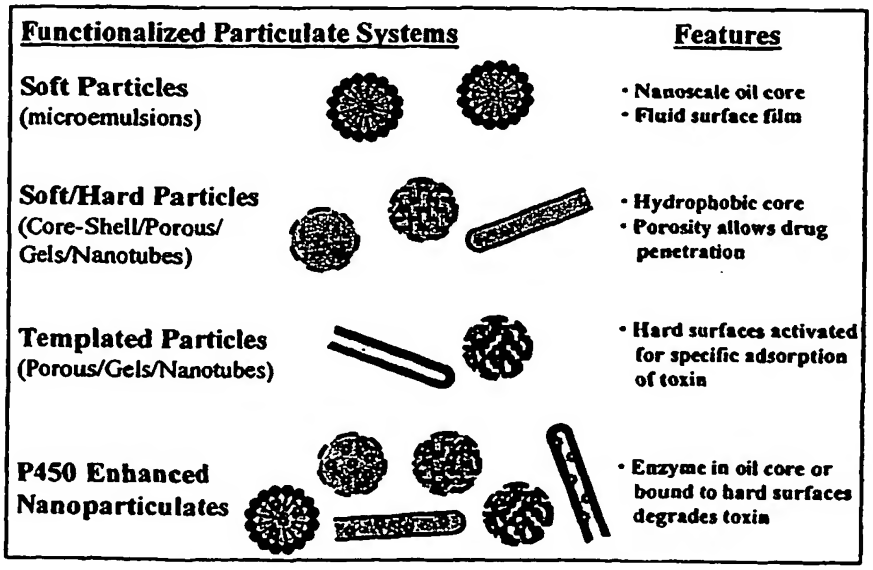
(54) **Title:** DETOXIFICATION AND DECONTAMINATION USING NANOTECHNOLOGY THERAPY



(57) **Abstract:** A method for detoxification and decontamination comprising the steps of: adding nanoparticle size bioparticles having at least one enzyme incorporated therein to a solution and biotransforming the target chemical into inactive metabolites.

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some potentially fatal cases of drug poisoning. By linking the toxic drug to albumin and using it as a hapten, high affinity antibodies with excellent specificity can be theoretically formed for use against a particular molecule or a class of molecules. The last several years have brought major innovations in the safety and efficacy of immunotoxicotherapy. In addition, advances have occurred in the processes of fragmentation which permits a greater volume of distribution (VOD) and diminished risk of sensitization and enhanced renal elimination.

Volume of distribution is defined as the volume of fluid that would be necessary to contain the amount of drug in the body at a uniform concentration equal to that in plasma. Thus, the definition assumes the body is a single homogeneous fluid compartment and the drug is evenly distributed throughout. The VOD may exceed the actual volume of the body. In application, VOD defines the concentration following one intravenous dose and roughly describes tissue penetration. A large VOD indicates good tissue penetration, while a small VOD indicates poor tissue penetration.

The binding portion of the antibody, known as the Fab fragment, has been found to reduce the physiological effects of drugs, such as digoxin, PCP, cocaine, colchicine, and tricyclic antidepressants in various animal models. However, despite these reported advances and the purported potential advantages of antibody use over other drug intoxication therapies, only one commercial antibody product is currently available to treat drug overdoses in humans. Digibind®, a digoxin immune Fab, is a lyophilized powder of antigen binding fragments (Fab) derived from specific antibodies raised from sheep. It has been shown to be highly effective in treating the life threatening cardiotoxic effects of digoxin, an inhibitor of the myocardial Na+/H+ ATPase pump.

A number of reasons explain the lack of widespread immunotoxicotherapy use in humans. First, there is no guarantee that a specific antibody can be produced that can effectively bind to a given target toxic drug molecule or its associated class of molecules. In addition, since

as toxicities arising from drugs having relatively high toxic blood concentrations.

Even for a drug like digoxin that causes drug toxicity at much lower concentrations than do drugs such as amiodarone and amitriptyline and is much less protein bound, the cost of treatment can still be prohibitive. For example, depending upon the blood concentrations of digoxin, 10-20 vials or more of Digoxin immune Fab (ovine), trade named Digibind®, may be required to effectively treat a 70 kg (155 pound) individual. This can translate to a cost of approximately $10,000 to $30,000. Accordingly, the cost of using an agent such as Digibind® can be prohibitively expensive where the potency of the drug is much lower and consequently a much greater amount of toxic drug (e.g., amiodarone or amitriptyline) would be required to be bound by the antibody.

Therefore, in the current state of development, antibodies are not a viable option to treat the vast majority of drug overdoses. Furthermore, even if new advances in molecular medicine allowed human-derived antibodies to be produced in mass quantities at a reasonable cost, their slow onset time of actions in humans is another limiting factor. For example, the median time to initial response for Digibind® is reported to be 19 minutes. Only 75% of patients showed evidence of response within 60 minutes. Slow response combined with inability to provide broad substrate detoxification and high cost are expected to continue to make antibodies a poor choice for the treatment of drug toxicity.

Another method for reducing toxic drug effects is infusion of enzymes into the blood. This method may be feasible to ameliorate toxic in-vivo effects of drugs. Specifically, the acute physiological effects of cocaine in various animal species have been reported to be acutely ameliorated using animal and human butyrylcholinesterase, the principal esterase in the blood that degrades cocaine to its major metabolites.

While sometimes effective, this approach suffers from a number of drawbacks. First, water-soluble plasma enzymes, such as

Second, the rate of removal of a drug from the blood must be taken into account. Hemodialysis and hemoperfusion are slow (e.g., generally hours) to generally remove drugs at toxic levels from the bloodstream.

For example, the intoxications of two testbed drugs, amiodarone and amitriptyline, are frequently life threatening because of their severe effects on cardiac function. Accordingly, treatment under these conditions must be initiated immediately or a patient may die. Consequently, hemodialysis or hemoperfusion cannot be used as a treatment for fast acting life threatening drugs such as amiodarone and amitriptyline. In addition, these approaches require the placement of large arterial and venous cannula prior to circulation of blood through the dialysis machine. As a result of its shortcomings, hemodialysis has minimal applications in treating drug toxicity. However, hemodialysis may be applicable for toxic drugs with low volumes of distribution and for those toxins that do not immediately produce life threatening effects.

Another method for treating drug poisonings is the use of specific pharmacological antidotes. However, of the many types of drug poisonings in humans, only a few have identified specific pharmacological antagonists that can be used to quickly and selectively reverse their deleterious physiological effects. Probably the best two examples of effective pharmacological antidotes are the muscarinic-cholinergic and narcotic receptor antagonists, atropine and naloxone, respectively. Atropine blocks the physiological effects of excessive acetylcholine levels on muscarinic receptors. Therefore, it is effective against organophosphate based insecticides as well as nerve gas agents.

In an analogous manner, naloxone blocks most of the physiological effects (e.g., respiratory depression) of narcotic overdosage. Therefore, it is effective in reversing the physiological effects of potent narcotics such as heroin and fentanyl. Although receptor antagonists are highly efficacious, rapid and specific in reversing these types of life threatening drug poisonings, they do so by preventing access of the agonist to its cellular locus of action (i.e., the receptor). Receptor antagonists neither alter the free blood drug

SUMMARY

A method for removal of at least one target chemical from a region includes the steps of adding a nanoparticle size bioparticle to the region and partitioning at least a portion of the target chemical into or onto the bioparticle. The method results in reducing the active concentration of the target chemical. The region can be a solution. Partitioning can result from differences in physicochemical properties between the bioparticle and the target chemical and/or from adsorption of the target chemical on a surface of the bioparticle.

A method for removal of at least one target chemical from a region includes the steps of adding a nanoparticle size bioparticle having at least enzyme incorporated therein to the region and biotransforming at least a portion of the target chemical into at least one substantially inactive metabolite. The region can be a solution. The enzyme can preferably be a genetically cloned enzyme.

A method for removal of at least one target chemical from a region includes the steps of adding a bioparticle having at least one enzyme incorporated therein and partitioning at least a portion of the at least one target chemical into or onto the bioparticle. The method results in a portion of the target chemicals being transformed into at least one substantially inactive metabolite. The region can be a solution. The bioparticle can be a nanoparticle. In one embodiment, the nanoparticle can include a silica nanotube having an alkyl compound attached to the silica nanotube. The bioparticle can have a size from approximately 1 to 100 nm or preferably from approximately 1 to 5 nm. Enzymes can include genetically cloned enzymes.

A method for treating a patient exposed to a toxic drug includes the steps of providing a plurality of nanosized bioparticles capable of mitigating the effects of the toxic drug through at least one mechanism selected from the group consisting of partitioning the toxic drug into or onto the bioparticle and transforming the toxic drug into at least one substantially inactive metabolite, and introducing the plurality of bioparticles and then introduced into the

detoxification (e.g., drug overdose), military (e.g., toxic warfare agents), industrial (e.g., manufacturing processes), environmental (chemical spill clean up), as well as many other purposes.

Drug poisonings may be treated by bioparticles which can use any or all of the following mechanisms:

1) partitioning a targeted drug onto the bioparticles by exploiting differences in physicochemical properties and/or using molecular templating to adsorb the drug onto functionalized surfaces of bioparticles;

2) biotransforming a targeted drug into an inactive metabolite(s). For example, an enzyme, such as a human purified and genetically cloned high activity enzyme, may be incorporated into a bioparticle to provide biotransformation effects on a targeted drug or toxin, or

3) preferably, providing a bioparticle which combines both approaches (1) and (2). These above methods are not limited to oral or intravenous use, but could also be employed to remove toxins from biological surfaces such as skin or nonbiological surfaces such as metal, wood or plastic. Moreover, the above methods can be used to detoxify a broad range of toxins.

The invention allows the synthesis of bioparticles that can directly reduce the free drug concentration in the blood, either by exclusively partitioning it inside the bioparticle, or more preferably, by partitioning and biotransforming the drug into an inactive metabolite within the bioparticle and ultimately promoting excretion from the body. With this approach, the principles of lipid partitioning and/or adsorption (via molecular templating) may act in a highly synergistic manner with those of biotransformation to provide drug detoxification systems with complementary detoxification mechanisms to provide added effectiveness. Thus, by providing a very high local concentration of toxic drug (substrate), partitioning and/or adsorption can dramatically increase the rate of enzymatic degradation depending upon the KM of the enzyme and its enzymatic efficiency it the bioparticle.

Nanoparticles (such as those shown in Fig. 1) have been created for treating a broad range of toxins. For example, "soft particles" such as

trapped. Examples are various metal oxides or solid organic polymers that have pores templated for neural amines, aromatic compounds or terpenes.

A shell containing pores can provide significant improve the effectiveness of nanoparticle-mediated drug detoxification. There are a number of potential design benefits of encapsulating either a hydrophilic or a hydrophobic environment with a solid shell containing nanopores. First, it could act to stabilize a nanoemulsion injected into the blood to prevent a dilutional effect on emulsion function. Second, it could act as a highly selective molecular filter by allowing only molecules of certain physical dimensions access to the bioparticle interior. Therefore, bioparticles could be targeted against at toxic molecules with molecular weights (MWs) at or below the selected cutoff points of molecular size. Third, by "trapping" locally high concentrations of coenzymes and cofactors important to CYP activity such as oxidoreductase (MW = 75,000) and cytochrome b5 (MW = 17,500) within the bioparticle interior and preventing their escape, a bioparticle with a shell incorporating nanopores would potentially enhance (or at least help preserve) optimal P450 enzymatic activity.

The reconstitution of P450 enzymes, especially the CYP 3A4 fraction, may be technically challenging with regards to preserving its catalytic activity, especially in blood. Inclusion of a shell can greatly aid this process. Fourth, a bioparticle shell with nanopores could prevent proteolytic degradation of CYP fractions while in the blood (i.e., armor the enzymes). It will allow ingress of smaller sized toxic drug molecules and easy egress of more water-soluble metabolites, but exclude those molecules greater than the pore size cutoff. In contrast, CYP fractions incorporated into soft bioparticles may not only be susceptible to degradation in blood, but their local concentrations of cofactors and coenzymes may not be preserved (see the second point given above). Fifth, a solid shell with nanopores could be designed to provide a biodegradable platform where the functional activity of the bioparticle is determined by the blood half-life of biodegradable shell. That is, once the job of biotransformation is complete, the biodegradable particle can slowly disintegrate and releases the

than 1 nor to as large as tens of micrometers. A correspondingly large range of nanoparticle sizes can be prepared. The template method is very versatile. It has been used to prepare nanoparticles composed of metals, semiconductors, other inorganic materials, carbons, etc. Nearly any method used to prepare bulk materials can be adapted to allow for synthesis of nanoparticles within the pores of a microporous template membrane. Significantly, a hollow tubular nanostructures can be obtained.

Membranes that have been used to prepare nanoparticles via the template method are shown in Figure 3. These are microporous aluminas prepared electrochemically from aluminum metal. The upper set of micrographs shows an inhouse prepared membrane of this type. In this case the pores are approximately 60 nm in diameter. The lower micrograph in Figure 3 shows a commercially-available membrane of this type. In this case the pores are 200 nm in diameter. These micrographs illustrate the important point discussed above that the pore diameter in such membranes (and correspondingly the diameter of the nanoparticle obtained). Ethylbutyrate ester was added at a low (ME-I) and high (ME-II) concentration. As seen in Fig. 2, the emulsion effectively sequestered significant quantities of amitriptyline, a tricyclic antidepressant agent with potential cardiotoxic effects, when compared to the saline control shown. The system labeled "Micelles" did not possess the oil core of ethylbutyrate ester which was possessed by both ME-1 and ME-II. By comparing the Micelles system to ME-1 and ME-2, it can be seen that the oil core significantly increased the amount of amitripyline adsorbed, the higher oil concentration (ME-II) adsorbing significantly more amitripyline than the lower oil concentration (ME-I). Drugs that may be amenable to this type of detoxification are not limited to tricyclic antidepressant agents such as amitriptyline, but may include drugs and toxins from all drug classes that have an affinity for, tend to combine with, or are capable of dissolving in lipids (lipophilic drugs).

Many types of oils, surfactants, and cosurfactants may be used to produce bioparticles based on nanoemulsion technology. The bioparticle

In the example shown in Fig. 2, a nanoemulsion was produced using Pluronic® L-44 (micelles) with or without addition of ethylbutyrate ester part of the nanotubule. Such template-synthesized nanoparticles can be composed of a wide variety of materials including metals, polymers, semiconductors, other inorganic materials, carbons, etc. The size of these nanoparticles (both the diameter and the length) can be controlled at will from the nm regime to the micrometer regime.

One embodiment of this technology is to use the template method to prepare hollow cylindrical silica nanoparticles. These nanoparticles are preferably prepared by using sol-gel template synthesis of silica within the pores of a microporous alumina template membrane. The silica tubules in this case were prepared using tetra ethylorthosilicate as the starting material; however, other precursors are available for preparing silica via the sol-gel method. Sol-gel silica nanotubules of this type have been prepared in the pores of various microporous alumina template of the type shown in Figure 3. It has been shown that both the inside and outside diameters and the length of the silica nanostructures can be controlled by varying the diameter of the pores and the thickness of the template used.

The tubular silica nanostructures prepared in this way can be derivatized both on their outside and inside surfaces with chemical and/or biochemical reagents. One approach for doing this is to use well-known silane chemistry. Hundreds of silanes are available commercially, so this is a very versatile route for chemically and biochemically derivatizing these silica nanostructures. This is important because such derivatization allows these nanotubules to extract or adsorb specific chemical reagents and allows them to catalyze specific biochemical reactions. In addition because the inside and outside of the tubules can be derivatized with different reagents, the inside and outside chemistry/biochemistry can be different. This is important because, for example, it might be desirable to have the interior of the nanotubules hydrophobic so that they will extract specific molecules but the

The 18-carbon alkyl (C_{18}) silane was chosen because this renders the insides of the nanotubules hydrophobic. The nanotubules with the C_{18} groups inside can then be used to extract hydrophobic molecules from a contacting solution phase. Again, in this case the outsides of the nanotubules remain hydrophilic silica and this allows these tubules to be dispersed into solutions containing polar solvents. The most obvious example is water, but the same principle applies for other polar solvents. Obviously, the outside could also be derivatized with the hydrophobic silane and such tubules could then be dispersed into solutions containing nonpolar solvents. Other alkyl silanes could be used to tune the extraction selectivity of the derivatized nanotubules. Examples include using shorter chain (e.g. C_{18}) alkyl silanes to make the tubules less hydrophobic on the inside, using aromatic silanes, using silanes with specific chemical functionalities (e.g., acidic or basic), etc.

The hydrophobic C_{18} silane-containing tubules were used to extract a hydrophobic target molecule (7,8-benzoqunoline or BQ) from a dilute aqueous solution. Extraction was accomplished in two ways. In the first method the hydrophobic nanotubules were left embedded within the pores of the template membrane, and a piece of the membrane was simply immersed into and then removed from the solution of the target molecule. Removing the membrane also accomplished the removal of the target molecule BQ sequestered inside. In the second method, the nanotubules were liberated from the template membrane, by dissolving the membrane in phosphoric acid solution. The liberated tubules were then collected by filtration. The tubules were then dispersed into a solution of the target molecule. The solution was then filtered to remove the tubules as well as the target molecule BQ sequestered inside. Figure 4 shows an example of the second method, dispersion of the liberated nanotubules. This figure shows first the UV absorption spectrum of a solution that was 1 x 10-5 M 7,8-benzoquinoline solution (BQ). To this solution was first added silica nanotubules that did not contain the hydrophobic C_{18} silane inside. (10 mg of tubules added per 100 mL of solution.) The solution was then filtered to remove these tubules and the solution spectrum was remeasured.

"blank membrane" (without nanotube incorporation of GOD) was determined to establish a baseline absorbance. Al_2O_3/SiO_2/GOD membranes were then immersed into the glucose solution. The time dependent changes in the absorbance (concentration) of glucose (assayed indirectly via oxidation of o-dianisidine) were determined. The results are depicted in Fig. 6. Based on the absorbance spectra shown, it is apparent that GOD incorporated into pores of the nanotubules converted much of the glucose in the solution to glucono-1,5-lactone upon immersion of the membrane (at approximately 140 seconds). As shown in Fig. 6. at approximately 400 seconds the membrane was removed from the solution. No further oxidation of glucose is observed because in removing the membrane the GOD incorporated inside is removed.

The nanotubes are important because they are new morphologies of particulate material. They are also important, being newly available, for evaluation and use in biomedical applications either by themselves or modified as described in this application. However, the nanotube is not the only shape carrier/core particle that can be derivatized as is described in this application. Many other shapes are useful, such as derivatized polyhedral-shaped porous (templated or not) nanoparticles.

Based on this observation, it was concluded that enzymes incorporated into nanoparticles can be used to degrade drugs. The linkage of enzymes to the inner surface of pores, can be achieved without losing the enzyme's reactivity. Although nanotubes having inner cavities for enzyme attachment was used, any shaped particle, whether tubular or not, having pores adapted for this purpose can have enzymes inside the pores. Thus, any nanoparticles having pores, whether the pores are tubular or any other shape may be used with the invention.

An identical approach (i.e., linking a cytochrome P-450 (CYP) enzyme system to the inner surface of a nanoparticle) can also be used to efficiently reduce the free concentration of lipophilic agents in human plasma and blood by a biotransformation dominated mechanism.

TABLE 1

Values of Shift for Donor-Trinitrobenzene Complexes in Chloroform-d

Donor	Shift (ppm, for acceptor)	Direction
2,6-Dimethylaniline	0.2995	Upfield
2,4-Dimethylaniline	0.3086	Upfield
3, 5-Dimethylaniline	0.2647	Upfield
3,4-Dimethoxytoluene	0.2730, 0.1868b	Upfield

notes: a [donor : acceptor] = 60:1 b at concentration in literature, lit. Value = 0.1830

TABLE 2

Values of Shift for Donor-N-Methyl-3,5-dinitrobenzamide Complexes in Chloroform-d

Donor	Shift (ppm, acceptor)	Direction
2,6-Dimethylaniline	Triplet 0.0874	Upfield
	Doublet 0.0779	Upfield
2,6-Dimethylacetanilide	Triplet 0.0156b, 0.1584	Upfield
	Doublet 0.00775b, 0.0055	Downfield,Upfield
Bupivacaine (salt)c	Triplet 0.0891	Upfield
	Doublet 0.0275	Upfield

notes: a [Donor : Acceptor] = 60 : 1, except case b where [D : A] = 1 : 1; c Studied in 50 : 50 D20 : CD3CN

rapidly attenuated bupivacaine induced prolongation of the QRS interval. Two additional experiments were also carried out, both yielding similar results.

Therefore, compared to available methods, bioparticles which may be produced using the invention have numerous advantages over current methods for treating drug toxicity. Advantages from the invention are enhanced through use of complementary approaches including lipid partitioning, adsorption and xenobiotic biotransformation.

Bioparticles using lipid partitioning and/or drug biotransformation produced using the invention not only scavenge most toxic drugs that are more lipophilic (active drug state normally) but also offer broader substrate usage. For example, various soft bioparticles can effectively reduce the free blood concentration of all virtually lipophilic drugs. Moreover, appropriately chosen enzymes incorporated into bioparticles can further improve the bioparticle's therapeutic performance and applicability by adding metabolization effects applicable to a broad range of drugs. If desired, the feature of chemical selectivity inherent in immunotoxicotherapy can be incorporated into bioparticles by using the processes of molecular templating and/or adsorption onto functionalized surfaces.

Using bioparticles, large lipid-water partition coefficients for, highly lipid soluble substances such as amiodarone indicate that the free concentration of this antiarrhythmic agent can be effectively reduced by using a concentration of soft bioparticles in the bloodstream that should not be detrimental to cell function (approximately 1.5% maximum). A bioparticle having a large lipid-water partition coefficient (e.g. 10,000),where the lipid component of the bioparticle is either liquid solid core, or lipophilic molecular entities attached to the surface of an inorganic core can bind a large fraction of highly lipophilic drugs. Thus, a drug's free blood concentration can be effectively reduced in a small volume of soft bioparticles.

In a preferred embodiment of the invention, bioparticles containing P450 cytochrome components such as a CYP 3A4 fraction are used to not only offer broad substrate detoxification, but also to produce rapid elimination of

gained by incorporating a lipid matrix within the bioparticle is that this structure may dramatically increase the intrinsic biocatalytic efficiency of an enzyme. Salt-immobilized hydrolytic and co-factor requiring enzymes (lyophilizates of enzyme in a salt matrix) in organic solvents have been shown to have 100-3000 times more activity than that observed in aqueous mediums (U.S. Patent No. 5,449,613 to Dordick, et al.). This technology applied to CYP fractions located within bioparticles can produce extremely efficient biocatalytical tools for drug detoxification, particularly when preferred high efficiently molecular cloned supersomes are used.

Numerous products can be produced from the invention including those composed of multiple types of nanoparticles for in-vivo detoxification of drugs and toxins from humans or animals. For example, nanoparticles can be synthesized for attenuating acute cardiotoxic effects of tricyclic antidepressant drugs (e.g., amitriptyline). However, the invention is not limited only to tricyclic anti depressants, but encompasses all drug classes that may cause toxicity. In addition, biological toxins (e.g., snake and insect envenomation) may also be detoxified using the invention. This can ensure human and animal safety and welfare. Furthermore, endogenous toxins produced during organ dysfunction or failure (e.g., hepatic or renal failure) may also be removable using the invention to create "circulating hepatocytes" or "circulation nephons."

Another potential product which can be produced from the invention is a product for detoxification of poison warfare agents that are used for military purposes (e.g., nerve gas). It is noted that warfare agents may be solids, liquids or gases. Warfare agents can cause massive intoxification of substances such as acetylcholine or tissue necrosis from direct toxicity (e.g., mustard gases). Rapid and simultaneous removal of both the warfare agent and molecules causing injury may prove to be effective therapy to mitigate the dangers of these weapons of mass destruction. For example, bioparticles produced using the invention could be used intravenously to reduce the concentration of both the toxin (e.g., sarin) and acetylcholine. Alternatively,

CLAIMS

We Claim:

1. A method for removal of at least one target chemical from a region, comprising the steps of: adding a nanoparticle size bioparticle to said region, and partitioning at least a portion of said at least one target chemical into or onto said bioparticle, whereby the active concentration of said at least one target chemical in said region is reduced.

2. The method of target chemical removal of claim 1, wherein said region is a solution.

3. The method for target chemical removal of claim 1, wherein said partitioning results substantially from differences in physicochemical properties between said bioparticle and said at least one target chemical.

4. The method for target chemical removal of claim 1, wherein said partitioning results substantially from adsorption of said target chemical on a surface of said bioparticle.

5. The method for target chemical removal of claim 1, wherein said partitioning results substantially from differences in physicochemical properties between said bioparticle and said at least one target chemical and said partitioning results substantially from adsorption of said target chemical on a surface of said bioparticle.

6. A method for removal of at least one target chemical from a region, comprising the steps of: adding a nanoparticle size bioparticle having at least one enzyme incorporated therein to said region, and biotransforming at least a portion of said at least one target chemical into at least one substantially inactive metabolite.

the effects of said toxic drug through at least one mechanism selected from the group of mechanisms consisting of partitioning said toxic drug into or onto said bioparticle and transforming said toxic drug into at least one substantially inactive metabolite, and introducing said plurality of bioparticles to said patient.

17. A method for treating an animal exposed to a toxic drug, comprising the steps of: providing a plurality of nanosized bioparticles capable of mitigating the effects of said toxic drug through at least one mechanism selected from the group of mechanisms consisting of partitioning said toxic drug into or onto said bioparticle and transforming said toxic drug into at least one substantially inactive metabolite, and introducing said plurality of bioparticles to said animal.

18. A composition for detoxification, comprising: a plurality of nanoparticles, said nanoparticles having at least one surface adapted for toxic drug attachment, said nanoparticles being at least one selected from the group consisting of microemulsions with nanoscale oil cores having soft surface films, hydrophobic cores having porous or soft shells and hard surfaces for specific adsorption of toxins.

19. The composition for detoxification of claim 18, further comprising attached enzymes for chemically degrading toxins.

20. The composition for detoxification of claim 19, wherein said attached enzymes includes genetically cloned enzymes.

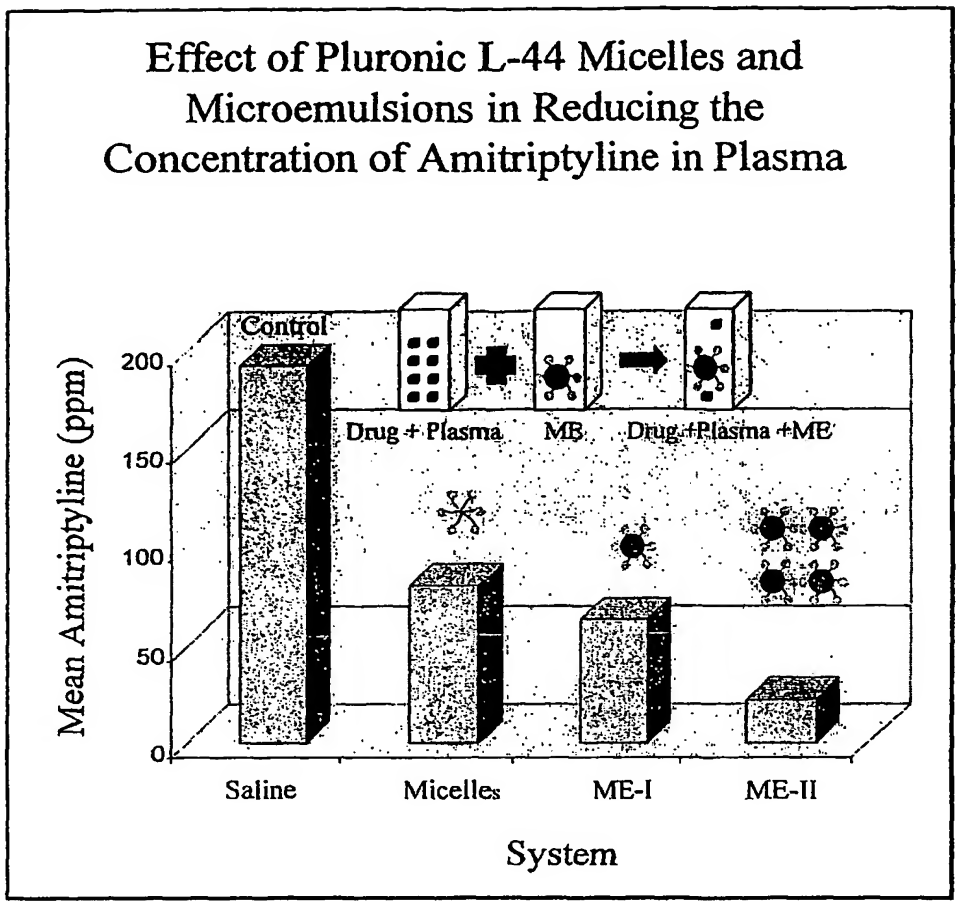

FIGURE 2.

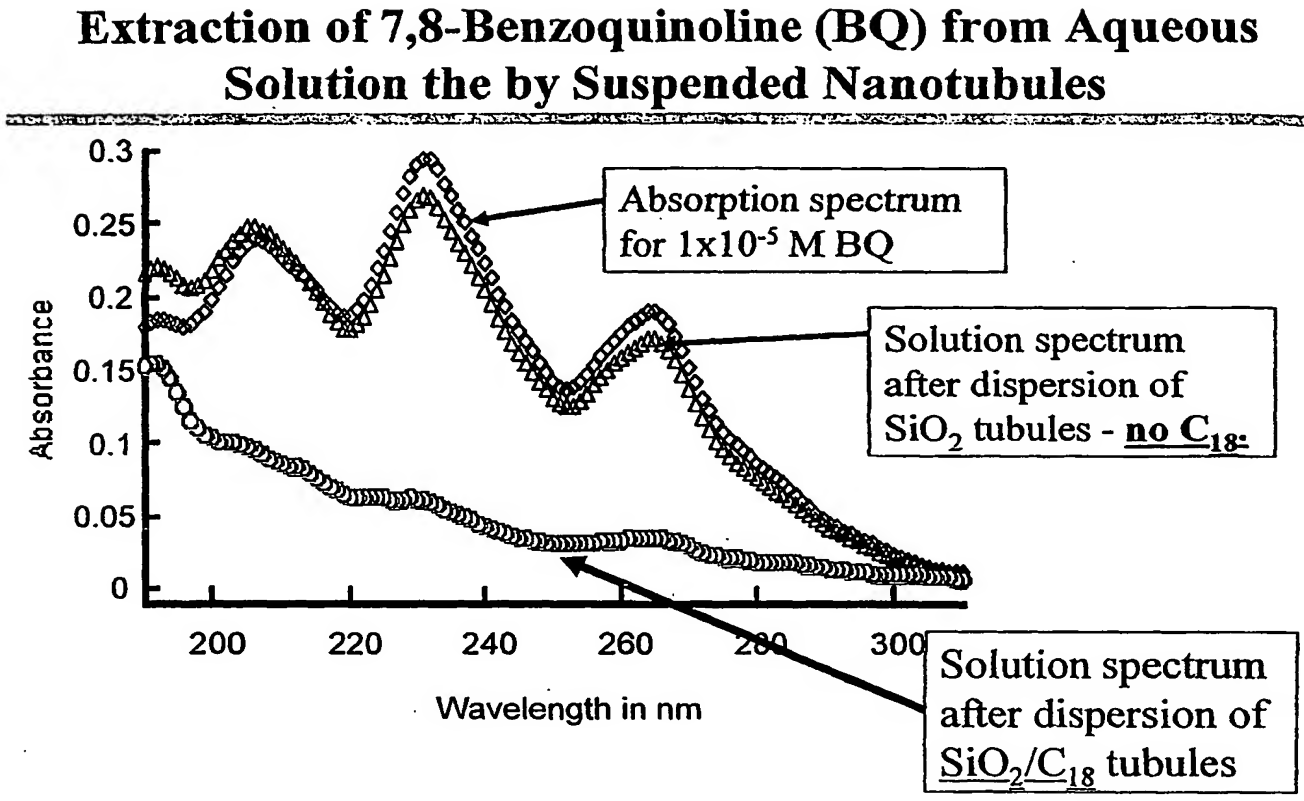

FIGURE 4.

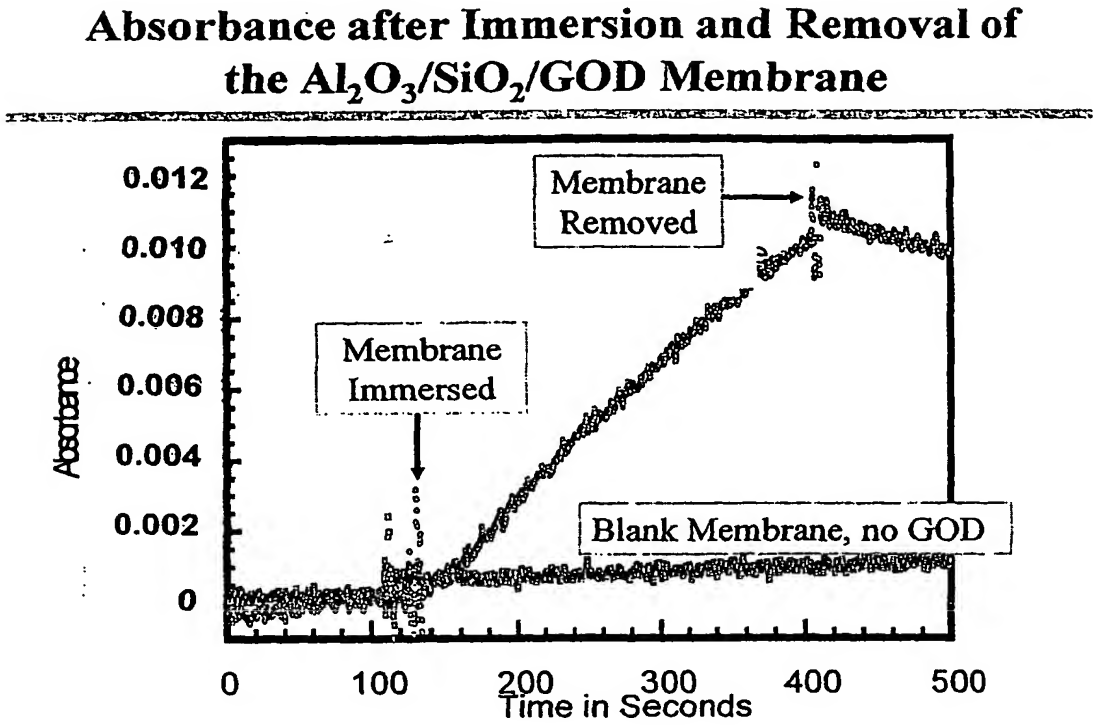

FIGURE 6.

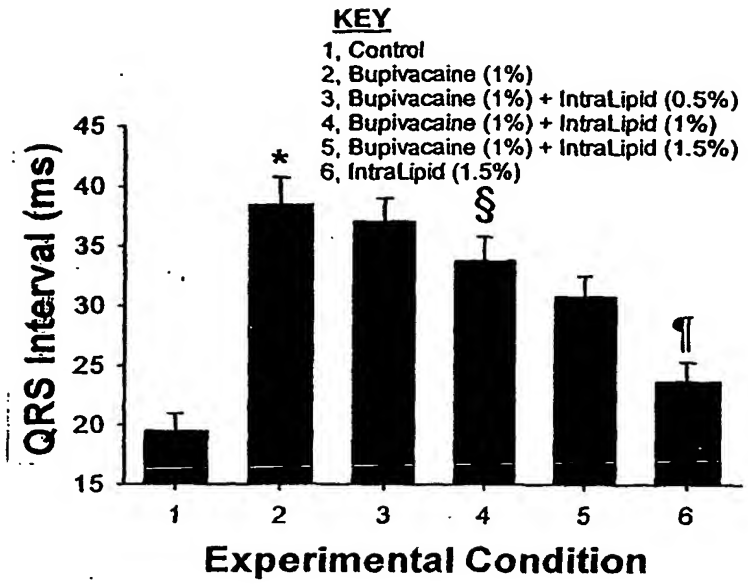

FIGURE 8.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 02/06114

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K9/14

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K A61L A62D C02F B01D A23L B09C

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
BIOSIS, EPO-Internal, WPI Data, PAJ, CHEM ABS Data, MEDLINE, FSTA

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	GILL IQBAL ET AL: "Degradation of organophosphorous nerve agents by enzyme-polymer nanocomposites: Efficient biocatalytic materials for personal protection and large-scale detoxification." BIOTECHNOLOGY AND BIOENGINEERING, vol. 70, no. 4, 20 November 2000 (2000-11-20), pages 400-410, XP002210869 ISSN: 0006-3592 the whole document -/-	6,7, 9-11,13

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
28 August 2002	16/09/2002

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Leutner, S

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

national application No.
PCT/US 02/06114

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

see FURTHER INFORMATION sheet PCT/ISA/210

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.